MODELS OF BIOLOGICAL REACTORS

By J. D. Norman

A mathematical model of a biological reactor should indicate the response of a given system to any conditions in which we are interested. In order to develop such a model we hope to utilize information from simple, well-defined systems. We must be cautious, however, when synthesizing a general model to ensure that information from the simple system is not amplified indiscriminately. To illustrate this point, a typical biological system will be considered and methods for developing an appropriate model will be discussed.

A typical biological system of interest is a continuous flow stirred tank fermenter with a separator and recycle of biological solids. Consider an input to this system of one component whose concentration is being reduced in the reactor, with the resulting production of new organisms, gaseous end products, and energy. Food + organisms ⟶ new organisms + gases. A flow diagram of such a typical system would be as shown in Figure 1.

Recycle of organisms is required in such a system because the rate of production of organisms may be less than the rate of washout from the reactor. Recycling of organisms can thus provide for rapid reduction of the food material even for large washout rates.

Balances may be written for each of the components in the reactor, in the usual way:

food
$$\frac{dC_A}{dt} = \frac{q}{V}\left(C_{A_0} + n \cdot C_A - C_A\right) - C_B R_A \qquad (1)$$

organisms
$$\frac{dC_B}{dt} = \frac{nq}{V}\alpha C_B - \frac{q}{V} C_B + C_B R_B$$
$$= \frac{q}{V}\left(n\alpha C_B - C_B\right) + C_B R_B \qquad (2)$$

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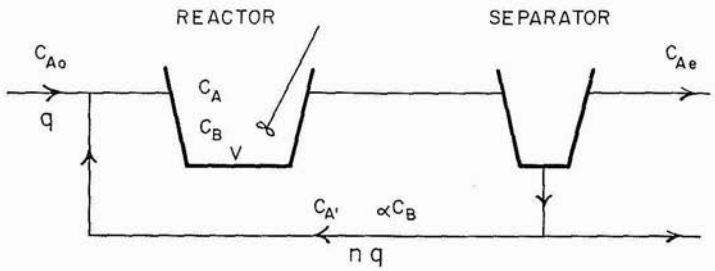

q = VOLUMETRIC FLOW RATE

C_A = CONCENTRATION OF FOOD

C_B = CONCENTRATION OF BIOLOGICAL ORGANISMS

FIGURE 1 - TYPICAL BIOLOGICAL REACTOR

Where R_A is rate of decrease of soluble organic material (food) and R_B is rate of formation of organisms both per unit mass of organisms in the reactor. The volumetric recycle rate is nq, and α is the concentration factor of C_B from the separator.

In biological systems of this simple form the relation which exists between R_A and R_B is not easily defined. The relation changes, depending on several environmental conditions.

Another important basic factor must be elucidated at this point. Although the example shown is for a simple biological system, the reaction terms (R_A and R_B) are also not readily defined in terms of the reactants.

In an analogous simple chemical reactor, description of the system performance is grossly simplified because rates of formation often can be expressed in the form $R = k(T)C$ where k is a function only of temperature.

Attempts to represent simple biological reaction rates as power products of concentration of substrate and organisms have not been successful. At least, such representations have not held for a general case, but may have been applicable over a narrow range of experimental conditions. To illustrate the point, we can look at the limiting cases. On one hand we have the "excess food case" where growth of organisms proceeds exponentially by binary fission such that $\frac{d C_B}{dt} = \gamma C_B$. This representation is simple and makes the solution of equations relatively direct, but only represents the situation in a reactor for a very limited period of time. An excess food situation cannot be maintained indefinitely. For instance, in the case of a batch study for rate data, one

organism with a generation time of twenty minutes would produce 2^{144} progeny in two days. The resulting mass would be some 4000 times the mass of the entire earth, hence prolonged periods of unrestricted growth are not easily attained.

In the other extreme, where food concentration is limited, net growth of organisms may be almost zero. Similarly, there are cases where essential growth nutrients are not provided, so that growth is restricted, yet the organisms maintain their metabolic ability to produce a desired product. In this case, the observed kinetic result is similar to that found with catalysts in chemical process where the mass is constant but the effectiveness decreases with use.

Most real-life conditions exist somewhere between these two limits. Although the reactions are concentration-dependent, reaction "constants" are highly dependent on the physical and biological environmental conditions.

Observations on batch reactions for single substrate systems show that growth rate is a maximum until the substrate concentration reaches some limiting value. Growth rate is expressed in "rate of utilization of substrate per unit mass of organisms." For batch studies this unit rate decreases steadily as substrate concentration nears depletion.

In an attempt to obtain similar growth rate and substrate utilization relations under continuous flow conditions, the "chemostat" was developed. This is a simple stirred reactor with no recycle of organisms. Hence, growth rates can be determined for a variety of residence times by adjusting input volumetric flow and feed concentration until a steady state is established in the reactor. The growth rates thus obtained were represented as a fraction of the maximum specific growth rate, μ_{max}, in $\frac{dC_B}{dt} = \mu_m C_B$ for the case of exponential growth. However, the information is often recorded as a function of reactor concentration only and does not take into account the volumetric flow rate or the feed concentration. A graphical plot of such data is shown in Figure 2. When this information was originally recorded, it was observed that the data could be fitted by a curve of the form:

$$\frac{\mu}{\mu_{MAX}} = \frac{C_A}{K + C_A} \tag{3}$$

which approximates an exponential function.

Since this expression had the same form as the Michaelis-Menton enzyme equation (see biochemistry paper) and since this was also a living system, the relationship for μ was accepted as a universal growth rate parameter. For many years, this has been used, without question, regardless of the application. The above expression not only fails to take into account the variables in a continuous flow system, but even fails to

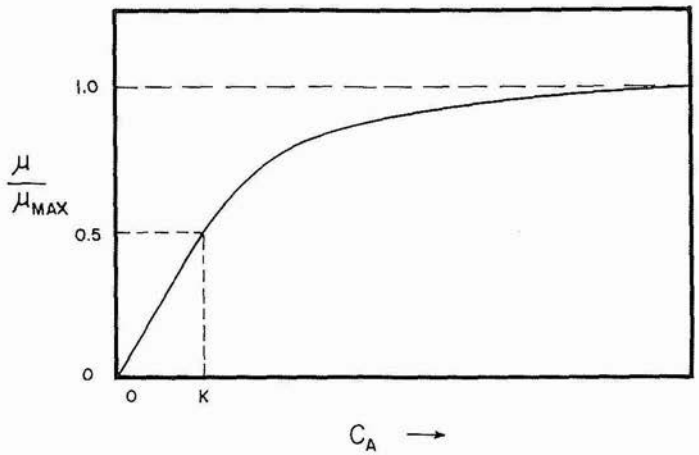

FIGURE 2 - TRADITIONAL GROWTH RATE CURVE

hold for all test conditions in a chemostat. Each growth rate value for a steady state in the chemostat represents a specific combination of inlet feed concentration and bulk flow rate.

This discussion has thus far concentrated mainly on delineating the methods currently accepted for describing biological reactions, and some of the reasons for their inappropriateness. Let us consider next the ways in which we may exploit techniques currently used in studying chemical reactors and examine how appropriate they might be.

1) Temperature effects: Although biological reactions show a degree of temperature dependence, only a relatively narrow range of operating temperatures is encountered. For most organisms 37°C is the upper limit. Ambient temperature is the practical lower operating limit. In general, reaction dependence between these limits may be no more significant than the response of substrate diffusion rates.

2) Energy balances: Although exact biochemical mechanisms have been explained for a great many reactions, and although energy exchange relationships are quite well defined for each step, very little use has been made of thermodynamic concepts in a macroscopic sense. It is not considered practical at this time, for instance, to obtain energy relationships experimentally from laboratory reactors due to measuring technique limitations at the concentrations employed. Errors in measurement may exceed actual energy changes. Thus the solution of equations is restricted without a set of energy relationships.

3) Batch studies: It is common with chemical reactions to obtain kinetic relations from a specific batch test and apply the information to

a general case for wide conditions of continuous flow situations. Biological systems, however, pose additional complications in this technique, because concentrations of both food and organisms vary with time. Further, the rate of change of each reactant is not a constant function of both reactants. Thus, it is not possible to establish an "order of reaction" per se, from a batch experiment.

Probablistic models have been employed in an attempt to describe the progress of biological reactions. These deal with the probability that organisms will divide within a given time span. For example, Θdt where $\Theta = \Theta\ (t,\tau, N)$ where τ is cell age and N the numbers of organisms. While probability functions offer an interesting approach, they appear to have limited use at present. One reason is the difficulty of confirmation of the theory due to measuring techniques. Another is the underlying assumption that all activities of an individual cell are a direct function of its physiological age. This is perhaps the greatest weakness in this approach.

Since several points have now been discussed on the inadequacy of existing methods of describing general biochemical reactions, it becomes apparent that new approaches must be sought. Thus we look to physical models to describe the responses of a biological reactor.

A suggested approach is to develop a "state" parameter for organisms in a given system. By "state" parameter is meant, in effect, a number of categories by which organisms can be classified according to growth rates or substrate utilization rates. Hence these categories would range from the maximum rate (that of exponential growth) down to the minimum rate at which there is almost no growth and substrate is utilized for energy by the existing organisms. (I. e., for each state there would be an R_{Ai} and R_{Bi} pair where subscript i designates the number of category.) This concept is shown graphically in Figure 3.

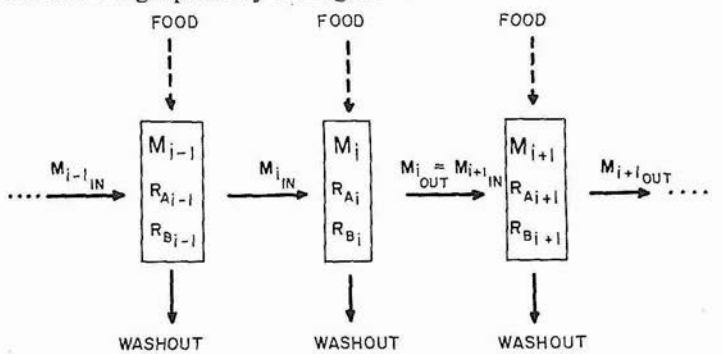

FIGURE 3 - SCHEMATIC OF REACTOR MODEL

Thus, if we know the mass of organisms in each category we could obtain good estimates of the over-all utilization of food and the over-all production of organisms. This can be obtained by a sequence of coupled mass-balance equations, providing we have a criterion which governs the movement of a given organisms from one category to the next.

In relating this view to the cases previously discussed (i.e., the extreme cases) it can be seen that this technique would incorporate all possible conditions of organisms in a reactor, independent of the flow rate or concentration.

For the extreme states we could say that when there is maximum unrestricted growth (i.e., exponential) all the organisms could be considered in the first group (or category). For the other extreme, when there is essentially zero growth and minimum substrate utilization (i.e., only for maintenance) all organisms would be in the Nth category. For intermediate conditions there would exist a large set of distribution functions to describe the distribution of the mass of organisms in each category.

In order to calculate, for any time, the mass of organisms in each category, a sequence of differential equations can be set up and solved simultaneously.

$$\begin{aligned}
\dot{M}_1 &= \dot{M}_{1_{IN}} - \dot{M}_{2_{IN}} - \frac{q}{V} M_1 \\
&\cdots\cdots\cdots\cdots \\
\dot{M}_i &= \dot{M}_{i_{IN}} - \dot{M}_{i+1_{IN}} - \frac{q}{V} M_i \qquad (4) \\
&\cdots\cdots\cdots\cdots \\
\dot{M}_n &= \dot{M}_{n_{IN}} - \dot{M}_{n+1_{IN}} - \frac{q}{V} M_n
\end{aligned}$$

What we need now is the criterion which makes a living mass move from one state to the next lower. It is reasonable to postulate that such a criterion could be related to the physiological age of the organisms and take into account the various concentrations of substrate under which it existed for all periods during its lifetime. Thus, time alone is not sufficient, but perhaps some function of real time and generation time would be appropriate. This is where the value of a physical model may be shown. Assumptions can be made as to the criterion which may control the movement of organisms from one "state" to another. The next step is to verify the model. The simplest method is to test its validity on existing data and for the existing parameters. Satisfying this condition does not in itself verify the model. However, if it is not negated then we look for experimental conditions that will negate it if the model

is not valid. If none of the conditions which we create will negate the model, then we are in a position to apply the model to producing information on the system.

Summary

There are two principal purposes for which a model of biological reactors may be used. The first is the general case of predicting an output of substrate and organisms on a continuous basis for any condition of inputs. The second case is for control of a biological system to provide a desired output for any given input. A model which satisfies these criteria will thus fulfill the basic requirements of being both realistic and useful.